How many Pygmy Marmoset (*Cebuella* Gray, 1870) species are there? A taxonomic re-appraisal based on new molecular evidence

Jean P. Boubli[a,b+], Maria N. F. da Silva[b], Anthony B. Rylands[c], Stephen D. Nash[c] Fabrício Bertuol[d], Mário Nunes[d], Russell A. Mittermeier[c], Hazel Byrne[a], Felipe E. da Silva[af], Fábio Röhe[d], Iracilda Sampaio[e], Horacio Schneider[e], Izeni P. Farias[d], Tomas Hrbek[d+]

[a] *School of Environment and Life Sciences, University of Salford, Salford, UK*

[b] *Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil*

[c] *Conservation International, Arlington, VA, USA*

[d] *Universidade Federal do Amazonas, Manaus, Amazonas, Brazil*

[e] *Universidade Federal do Pará, Belém, Pará, Brazil*

[f] *Instituto de Desenvolvimento Sustentável Mamirauá, Tefé, Amazonas, Brazil*

[+] Corresponding author: j.p.boubli@salford.ac.uk, School of Environment and Life Sciences, University of Salford, Salford M5 4WT, UK: +44 (0)161-295-6825; hrbek@evoamazon.net, Department of Genetics, Federal University of Amazonas, Manaus, AM 69077, Brazil: +55 92 98419-4226

Abstract

The pygmy marmoset, *Cebuella pygmaea*, the smallest of the New World monkeys, has one of the largest geographical distributions of the Amazonian primates. Two forms have been recognized: *Cebuella pygmaea pygmaea* (Spix, 1823), and *C. p. niveiventris* Lönnberg, 1940. In this study, we investigated if the separation of pygmy marmosets into these two clades can be corroborated by molecular data. We also examine and compare coloration of the pelage in light of the new molecular results. We analyzed the mtDNA cytochrome *b* gene and, for the first time for any Neotropical primate, we used a reduced representation genome sequencing approach (ddRADseq) to obtain data for recently collected, geographically representative samples from the Rio Japurá, a northern tributary of the Rio Solimões and from the Javarí, Jutaí, Juruá, Madeira and Purus river basins, all tributaries south of the Solimões. We estimated phylogenies and diversification times under both maximum likelihood and Bayesian inference criteria. Our analysis showed two highly supported clades, with intraclade divergences much smaller than interclade divergences, indicating two species of *Cebuella*: one from the Rio Japurá and one to the south of Solimões. The interpretation of our results in light of the current taxonomy is not trivial however. Lönnberg stated that the type of Spix's pygmy marmoset (type locality 'near Tabatinga') was obtained from the south of the Solimões, and his description of the distinct *niveiventris* from Lago Ipixuna, south of the Solimões and several kilometres east of Tabatinga, was based on a comparison with specimens that he determined as typical *pygmaea* that were from the upper Rio Juruá (south of the Solimões). As such it remains uncertain whether the name

pygmaea should be applicable to the pygmy marmosets north of the Rio Solimões (Tabatinga type locality) or south (near Tabatinga but across the Solimões). Finally, our analysis of pelage coloration revealed three phenotypic forms: 1) south of the Rio Solimoes, 2) Eirunepé-Acre, upper Juruá basin; and 3) Japurá. More samples from both sides of Solimões in the region of Tabatinga will be necessary to ascertain the exact type locality for Spix's *pygmaea* and to resolve the current uncertainties surrounding pygmy marmoset taxonomy.

Key words: Amazonia, *Cebuella*, phylogenomics, taxonomy

58 **1. Introduction**

59

60 Weighing around 119 g (range 85−140 g; Soini, 1993), the pygmy marmoset is
61 the smallest of the New World monkeys. In spite of its diminutive size, it has one of
62 the largest geographical distributions for a single Amazonian primate (Fig. 1). Pygmy
63 marmosets are sister to the Amazonian marmosets (*Mico*). Molecular genetic data
64 have indicated that the two genera diverged during the early Pliocene (Perelman et al.,
65 2011; Schneider et al., 2012; Buckner et al., 2015), but, based on fossil material
66 studied by Marivaux et al. (2016), the divergence would appear to have been earlier,
67 in the early Late Miocene about 11 Ma. Pygmy marmosets are not sympatric with the
68 Amazonian marmosets; the Rio Madeira, a right bank tributary of the Amazon,
69 separates *Cebuella* from *Mico*, the latter restricted to the east and the former to the
70 west of the river. Pygmy marmosets are small exudativore-insectivores, sharing with
71 all other marmosets (*Mico* and *Callithrix*) dental adaptations to exploit tree gums; a
72 food that makes up a significant portion of their diet (Kinzey et al., 1975; Ramirez et
73 al., 1977; Soini, 1982).

74 The pygmy marmoset was first described by Johann Baptist von Spix in 1823,
75 with the type locality "near Tabatinga, Rio Solimões, Amazonas, Brazil." Tabatinga is
76 a small town on the north (left) bank of the Rio Solimões (upper portion of the
77 Amazon), near the Colombian border. In 1940, Einar Lönnberg described a
78 subspecies from the Lago Ipixuna, on the south bank of the Rio Solimões, which he
79 distinguished as having a paler and more greyish back than *C. p. pygmaea*, and
80 underparts (chest, belly, and inner surface of arms and legs) that were entirely white.

Although the geographic ranges of the two subspecies were poorly defined—*niveiventris* was known only from its type locality—this taxonomy was subsequently recognized by Cruz Lima (1945), Cabrera (1957), Vieira (1955), Hill (1957), Napier and Napier (1967), and Napier (1976) (Fig. 1). Hershkovitz (1977), however, did not accept the validity of *C. p. niveiventris*, concluding that the color of the underparts is individually and locally variable. Hershkovitz's (1977) conclusion that *C. pygmaea* was, *contra* Lönnberg, monotypic was accepted by Groves (1989), but Van Roosmalen and Van Roosmalen (1997) argued again for the validity of Lönnberg's subspecies, based on pygmy marmosets with white underparts that they found in localities on the west bank of the lower Rio Madeira. Groves (2001, 2005) concluded that the color distinction of the underparts *was* consistent and sufficiently diagnostic to justify the separation. Based on this alone, Van Roosmalen and Van Roosmalen (1997) proposed that the Rio Solimões was a barrier to the dispersal of the two taxa, separating *pygmaea* to the north and *niveiventris* to the south. Rylands et al. (2009) pointed out, however, that the situation was more complicated than that. Lönnberg (1940) had distinguished *niveiventris* by comparing it to what he believed was typical *C. pygmaea* Spix, from the upper Rio Juruá, south, not north, of the Solimões.

In this study, we use a molecular phylogenetics approach to clarify the current taxonomy of pygmy marmosets, taking advantage of the availability of newly collected samples from the Japurá, Javarí, Jutaí, Juruá, Madeira and Purus river basins, and thus covering distant parts of the species' distribution in Brazil, allowing us to further assess the diversity of this lineage. We followed previous studies that have used mtDNA markers to investigate the relationships of pygmy marmosets but also took a genomic approach (ddRADseq); the first use of genomics to investigate

105 the phylogenetic relationships of a group of New World primates. We also examine
106 and compare the pelage of these newly collected specimens with material studied by
107 Lönnberg deposited in Stockholm's Natural History Museum.
108
109 **2. Methods**
110
111 For the molecular analysis we used recently collected, geographically and
112 phenotypically representative samples of *C. pygmaea* from the Japurá, Javarí, Jutaí,
113 Juruá, Madeira, and Purus river basins, totaling 14 individuals (Table 1). For
114 outgroups, we used the genus *Aotus*, representing the monotypic Aotidae, and 12
115 additional species representing all callitrichid genera except for the lion tamarins
116 *Leontopithecus*. We chose outgroups to provide multiple calibration points, sister-
117 species pairs, and species pairs that are separated by the Rio Amazonas-Solimões
118 (Table 1). For all specimens, we generated new sequence data, except for *Callithrix*
119 *jacchus* and *Aotus nancymaae* where the complete mitochondrial cytochrome *b* gene
120 and homologous genomic regions were extracted from the complete genomes
121 deposited in Genbank.
122 Total genomic DNA was extracted from muscle tissues preserved in 95% ethanol
123 using the standard phenol-chloroform extraction protocol of Sambrook et al. (1989).
124 We amplified the complete mitochondrial cytochrome *b* (cyt *b*) gene by polymerase
125 chain reaction (PCR) with the primers MonkeyGluF1 (5'-
126 CCATGACTAATGATATGAAAARCC-3') and MonkeyProR1 (5'-
127 AGAATSTCAGCTTTGGGTGTTG-3') developed for this study. PCR products were
128 purified using ExoSap (Werle et al. 1994) and subjected to fluorescent dye-terminator

129 (ddNTP) sequencing following the manufacturer's recommended protocol for BigDye
130 sequencing chemistry (Applied Biosystems) and using the primers MonkeyCytbF2
131 (5'-GGATCAARYAAYCCRTCAGG-3'), MonkeyCytbR1 (5'-
132 GCBCCTCAGAADGATATTTG-3') and MonkeyCytbR2 (5'-
133 CGTAGRATTGCRTATGCRAA-3') developed for this study. Subsequent to the
134 cycle sequencing reaction, the products were precipitated with 100% Ethanol / 125
135 mM EDTA solution, re-suspended in Hi-Di formamide, and resolved on an ABI
136 3130xl automatic sequencer (Applied Biosystems). Sequences were assembled,
137 edited, aligned and trimmed using the software Geneious v8.1.8; alignment was done
138 using the MUSCLE (Edgar, 2004) alignment plugin in Geneious v8.1.8 and
139 confirmed by eye.
140 We also performed a partial representational genome sequencing using the double
141 digest RAD sequencing protocol (ddRADseq) (Peterson *et al.*, 2012). This protocol
142 was adapted to allow simultaneous digestion and adaptor ligation, and for use on the
143 IonTorrent PGM (https://github.com/legalLab). Briefly, 200 ng of genomic DNA of
144 each individual was digested with SdaI and Csp6I restriction enzymes (ThermoFisher)
145 and the IonTorrent P and A adapters were linked to the digested fragments, all in one
146 step. The fragments were enriched via PCR. The A adaptor is a "Y divergent" (Coyne
147 et al. 2004), resulting in the enrichment of only those ddRAD fragments with one P1
148 and one A adaptor. Furthermore, the A adaptor contains a unique molecular barcode
149 for identification of individuals. Following the PCR enrichment, we selected
150 fragments in the range of 320 to 400 bp using the Pippin Prep (Sage Science). Based
151 on the analysis of complete primate genomes deposited in Genbank, we expected to
152 observe ~12,000 ddRAD fragments in the range of 320 to 400 bp. This information

153 was then used to optimize the number of individuals to be analyzed in one run of the
154 IonTorrent PGM.
155 Finally, we developed a Python script to perform an *in silico* double digest of the
156 complete genomes of *Callithrix jacchus* and *Aotus nancymaae* available in Genbank.
157 Using this script we extracted all fragments in the size range of 320 to 400 bp
158 generated by *in silico* restricting the genomes with the SdaI and Csp6I restriction
159 enzymes whereby each fragment had to have both restriction enzyme cuts. The
160 complete ddRADseq protocol, scripts for estimating number of ddRAD fragments
161 within a given size range, and scripts for extracting ddRAD fragments from published
162 genomes are available on GitHub (https://github.com/legalLab).
163 Sequencing reads were processed using the pyRAD pipeline (Eaton, 2014).
164 During *de novo* assembly, we used a minimum coverage of 6x per locus/allele,
165 assembling all fragments in the 320 to 400 bp range. Following demultiplexing and
166 extraction of loci using the above criteria (steps 1−2 of the pyRAD pipeline), we
167 included the ddRAD loci of *Callithrix jacchus* and *Aotus nancymaae* extracted from
168 complete genomes, and proceeded with clustering of loci across individuals, and the
169 generation of datasets for analyses (pyRAD steps 3−7). In the final datasets a locus
170 was included only if it was present in at least 50% of individuals.
171 We jointly estimated phylogeny and diversification times under an uncorrelated
172 lognormal relaxed clock model implemented in the program BEAST v1.8.2 using soft
173 calibrations obtained from Perelman et al. (2011). We used normal priors with
174 standard deviations corresponding to those reported in Table 2 of Perelman et al.
175 (2011). Additionally, we cross-validated our topology under both the maximum
176 likelihood and Bayesian inference criteria in RAxML (Stamatakis, 2014) and

ExaBayes (Aberer et al., 2014), respectively. Robustness of the RAxML analyses was accessed via 1000 bootstrap replicates, while in the ExaBayes analyses we ran two independent chains each 3 million generations long, sampling 9000 topologies following burnin of first 10% of the trees. We also cross-validated our divergence time estimates using the *chronos* function in the R package APE (Paradis et al., 2004), using the mean calibration points from Perelman et al. (2011), and the 9000 topologies generated in ExaBayes.

We examined the coloration pattern of the pelage of all specimens used in this study and compared them with photos of the specimens used by Lönnberg to describe *C. p. niveiventris* as distinct from *C. pygmaea pygmaea*.

This research adhered to Brazilian laws that govern primate research as well as the American Society of Primatologists' principles for the ethical treatment of primates. Research permits were granted through relevant Brazilian authorities (FUNAI and IBAMA/ICMBio, and institutional IACUC committees—permit numbers are listed in the acknowledgments).

3. Results

3.1. Phylogenetic study

We sequenced the complete mitochondrial cytochrome *b* gene (1,148 bp) from 24 specimens, and extracted from Genbank the complete cytochrome *b* gene of *Callithrix jacchus* and *Aotus nancymaae*. Next generation sequencing on the IonTorrent PGM produced between 30 and 50 thousand usable reads per species after processing in pyRAD (Eaton, 2014). On average, between 7.5 and 14.3 thousand unique loci with

201 an average of 3.5x coverage were observed in each individual in the 320–400 bp
202 range; the number of loci obtained by data-mining the genomes of *Callithrix jacchus*
203 and *Aotus nancymaae* was 14170 and 14201, respectively. Filtering alleles and loci to
204 a minimum 5x coverage, between 850 and 1400 loci with an average coverage of 7.3–
205 8.3x coverage were retained. The probability of observing a spurious allele was less
206 than $1e^{-21}$. In the final dataset, where a locus had to be present in at least 50% of the
207 individuals (Streicher et al., 2016), 954 loci were retained. These 954 loci represented
208 954 unlinked SNPs and had 20891 variable sites of which 9764 were parsimony
209 informative. Total alignment length was 307673 bp.
210 Analysis in BEAST of the cytochrome *b* as well as the ddRADseq alignments
211 resulted in robustly supported phylogenetic hypotheses (Figs. 2 and 3) that were
212 identical except for phylogenetic relationships among *Cebuella p. pygmaea* and *C. p.*
213 *niveiventris* individuals (Figs. 2 and 3). Divergence time estimates were also similar
214 in the mtDNA and ddRAD phylogenies, however, in general, intermediate divergence
215 times were older in the mtDNA vs. the ddRAD phylogenies. Phylogenies generated
216 under the maximum likelihood and Bayesian inference paradigms implemented in
217 RAxML and ExaBayes, respectively, were identical, and were identical to those
218 inferred from BEAST (Drumond et al 2012) analyses. Divergence time estimates
219 inferred in BEAST and using the *chronos* function in the R package APE (Paradis et
220 al., 2004) was also comparable.
221 Notably, all phylogenetic analyses showed a split between pygmy marmosets
222 from the Japurá and those from the south bank of the Rio Amazonas-Solimões. These
223 two clades were highly supported with BI = 1.0 and ML bootstrap = 100. The
224 estimated divergence time between these two clades was 2.2452 (95% HPD 1.7059–

225 2.9606) MYA, while the divergence time between the sister taxa *Saguinus bicolor* and
226 *S. martinsi* was estimated at 0.8191 (95% HPD 0.5240–1.1150) MYA. Since some
227 authors consider *Saguinus martinsi* to be a subspecies of *S. bicolor*, we also estimated
228 the divergence of *Saguinus bicolor* clade from the *Saguinus midas* sister clade at
229 1.6319 (95% HPD 1.1964–2.2265) MYA. Although they are not necessarily sister
230 taxa (Buckner et al., 2014), the estimated time of divergence between *Mico*
231 *humeralifer* and *M. argentatus* distributed on the left and right banks of the Rio
232 Tapajós, respectively, was estimated at 1.1889 (95% HPD 0.8142–1.6892) MYA. We
233 also included night monkeys of the genus *Aotus*. The genus is composed of two main
234 clades, the gray- and the red-necked species occurring north and south of the Rio
235 Amazonas-Solimões, respectively. The distribution of these groups parallels that of
236 the supposed (see below) distribution of *Cebuella* in having distinct forms either side
237 of the Rio Amazonas-Solimões. The divergence of the *Aotus vociferans* clade from
238 the *A. nigriceps/nancymaae* sister clades found north and south of the Rio Amazonas-
239 Solimões, respectively, was estimated at 2.2584 (95% HPD 1.6370–3.0906) MYA.
240
241 *3.2. Pelage coloration*
242 With regard to overall pelage coloration, we identified three broad patterns for
243 specimens in the Brazilian Amazon: 1) south of the Rio Solimões, 2) Eirunepé-Acre,
244 upper Juruá basin, southwestern Brazilian Amazon; and 3) the Japurá basin, north of
245 the Rio Solimões (see Fig. 4 and Table 2 for full descriptions). To summarize, the
246 first, along the south bank of the Rio Solimões-Amazonas has white, pale, buff to
247 whitish undersides, including the inner arms and legs. The throat and chest are
248 ochraceous orange, but in some the throat is buffy to whitish in the centre. We are

249 provisionally referring to them as *C.* cf. *niveiventris* Lönnberg (Figs. 5 and 6). In the
250 second pattern, from the upper Juruá extending west into Peru and Ecuador, the
251 underparts are darker, dull yellowish-brown tawny, with a dull ochraceous orange to
252 brownish throat (Fig. 7). Lönnberg (1940) believed that these pygmy marmosets were
253 typical *pygmaea*. Our current molecular data suggest however, that they are
254 phylogenetically part of the same large clade containing all the remaining white or
255 pale chested specimens from south of the Solimões included in our analysis (although
256 they do form a separate subclade in the ddRAD tree, see Figs. 2 and 3). We are
257 provisionally referring to them as *C.* cf. *pygmaea* (Spix). The third pattern, from the
258 middle Japurá, north of the Rio Solimões in Brazil, have dark, orangey brown
259 underparts, and we are provisionally referring to them as *C.* aff. *pygmaea* (Spix)
260 (Figs. 5, 8 and 9).
261
262 **4. Discussion**
263
264 *4.1. Phylogenetic study*
265 The results of both our time trees showed two distinct, reciprocally monophyletic
266 groups of *Cebuella*; one on the right bank of the Japurá and the other south of the Rio
267 Amazonas-Solimões. They diverged from one another in the Pliocene/Pleistocene
268 boundary (2.2452 [95% HPD 1.7059–2.9606] MYA). Our time trees also show
269 divergence times of selected outgroup sister taxa or clades, helping put the split
270 between the two *Cebuella* clades into a broader perspective within the platyrrhines.
271 We recovered a more recent divergence time between the sister taxa *Saguinus bicolor*
272 and *S. martinsi* (Middle Pleistocene – 0.8191 [95% HPD 0.5240–1.1150] MA

divergence) and between *Mico argentatus* and *M. humeralifer* found on the opposite banks of the Rio Tapajós (Middle to Early Pleistocene – 1.1889 [95% HPD 0.8142–1.6892] MYA). *Mico argentatus* and *M. humeralifer* are unlikely to be sister taxa (Buckner et al. 2014), and thus, sister taxa divergences within the genus *Mico* are likely to be even more recent. The two *Cebuella* clades diverged at around the same time as the dwarf marmoset *Callibella humilis* from the Amazonian marmosets of the genus *Mico*. The divergence between the two *Cebuella* sister clades is older than that of any other callitrichid sister-species pairs (see also Buckner et al., 2015). Last but not least, the separation and timing of the separation of the *Cebuella* sister clades is paralleled by species of the genus *Aotus* where the grey- and the red-necked species clades occur north and south of the Solimões-Amazonas, respectively. These clades also diverged at the Pliocene/ Pleistocene boundary (2.2584 MA; 95% HPD 1.6370–3.0906 MA), and thus it is highly probable that the same historical event, such as the formation of the modern course of the Rio Solimões-Amazonas, resulted in the isolation of primate populations on either side of the river, eventually leading to vicariant speciation.

4.2 Pelage coloration

Hershkovitz (1977) concluded that variation in pelage coloration did not allow for the identification of more than one taxon of the pygmy marmoset, a conclusion that was unsettling considering its very small size and its very large geographic distribution. As is frequently the case, Hershkovitz was hampered by too few specimens (many of them of unknown or indefinite provenance), making geographic

297 patterns difficult or impossible to detect. This is exacerbated in the case of *Cebuella*
298 by the uncertainty concerning the type locality of Spix's *pygmaea* (in particular
299 regarding from which side of the Rio Solimões the type was collected), and the fact
300 that it is a mounted individual in poor condition, hindering its comparison with other
301 specimens (Elliot, 1913; and Fig. 8).
302 The type locality is "vicinity of Tabatinga on the banks of the River Solimões,"
303 Tabatinga is a town on the northern (left) bank of the Solimões (locality 3, Fig. 1),
304 and Cruz Lima (1945) complemented the type locality description pointing this out.
305 The south bank of the Solimões is also, however, in the vicinity of Tabatinga, and
306 Lönnberg (1940, pp.21−22) stated that "The type locality of the main species is
307 indicated by Spix to be Tabatinga, on the southern side of Rio Solimões near the
308 junction with Rio Javary", presumably near the town of Benjamin Constant.
309 Unfortunately, Lönnberg did not say where Spix provided this more precise type
310 locality. We checked the account of the Spix and Martius expedition (Spix and
311 Martius, 1938), but found no more specific reference to the type locality.The travel
312 diary refers mostly to Spix's interaction with the local Tikuna tribe and the time spent
313 with them. Given it is common for indigenous peoples to keep pygmy marmoset as
314 pets, it is likely that the type of *pygmaea* was a pet living in one to the villages visited
315 by Spix. In this case, determining the precise location where the type specimen
316 originally came from will be difficult, although Lönnberg must, of course, have had
317 access to a written source.
318 Van Roosmalen and Van Roosmalen (1997) attributed all pygmy marmosets north
319 of the Rio Solimões to *C. p. pygmaea*, even though Lönnberg (1940) in describing
320 *niveiventris* as a distinct subspecies had compared it with six specimens that he

deemed typical *C. pygmaea* from the upper Rio Juruá. south of the Rio Solimões. They were collected by A. M. Olalla from João Pessoa, Rio Juruá, in 1936 (one female collected on July 15, and two males and three females collected on July 24, 1936); today in the Natural History Museum, Stockholm. João Pessoa, also listed by Cruz Lima (1945), Vieira (1955), Hill (1957) and Cabrera (1957), is today called Eirunepé, and is on the left bank of the upper Rio Juruá 6°39'36"S, 69°52'26"W), opposite its confluence with the Rio Tarauacá (Paynter and Traylor, 1991) (see Fig. 1, locality 17). Lönnberg (1940) believed that the six specimens from there conformed with the description by Spix (1823) of *Cebuella pygmaea*:

> "The colour of these specimens agrees with the original description given by Spix, and repeated by later authors. The lower side is in these specimens dirty yellowish brown, sometimes with an irregular and narrow stripe of greyish or even dirty whitish extending some way along the middle of the belly" (pp.20−21).

Lago Ipixuna, the type locality of *niveiventris*, is approximately 700 km north-east of Eirunepé, on the south bank of the Rio Solimões, just west of the Rio Coarí, and about midway between the rios Tefé and Purus. Hence the vernacular names of western (*pygmaea*) and eastern (*niveiventris*) pygmy marmosets used by Hill (1957) and Napier (1976).

Van Roosmalen and Van Roosmalen (1997) attributed all pygmy marmosets south of the Rio Solimões to *niveiventris* based on five localities along the west (left) bank of the lower Rio Madeira (four sightings in the wild and a pet):

343 "All animals observed in the wild, as well as one live specimen obtained
344 in the community of Democracia, showed the typical characters of the
345 *niveiventris* subspecies" (p.4).

346 Van Roosmalen and Van Roosmalen's (1997) "typical characters", were "sharply
347 contrasting whitish chest, belly and inner surface of arms and legs." (p.4).

348 Hershkovitz (1977, p.464) reported on pygmy marmosets captured "near
349 Tabatinga" in 1963 that were received by the California National Primate Research
350 Center, Davis. The marmosets were short-lived, and their skins and skulls are now in
351 the U. S. National Museum (Smithsonian Natural History Museum), Washington, DC.
352 Twenty-four individuals (NMNH 336302-339325) caught from 23 February to 8 April
353 1963 mostly had buffy underparts, but the color varied from nearly white to orange. In
354 fact, all but two of the specimens are very similar in pelage coloration; all have buffy
355 (greyish or off white) underparts and distinct black speckling on the back and rump,
356 and a reddish-brown tail with black rings (see Fig. 10). Specimen 336302 (female)
357 was noticeably whiter on the chest and abdomen and inner legs and arms than the
358 others (Hershkovitz's nearly white underparts) (Fig. 11). The dorsum of specimen
359 336304 (female) is more uniform brownish with little black speckling, and the nape
360 and mantle were the same colour as the back to the rump. Its ventrum is closer to a
361 pale ochraceous (Hershkovitz's orange underparts) (Fig. 12). This "batch" may well
362 have originated from three localities (336302 and 336304 perhaps as pets purchased
363 in Tabatinga), but where exactly, and which side of the Rio Solimões, has not been
364 recorded.

365 A second batch of 12, captured in June and July 1963 (NMNH 337319-337330),
366 were all similar, with underparts that were mainly tawny but some more pale buff and

orange colored (Fig. 13). Hershkovitz observed that "the difference in color of underparts between the two batches is striking" (p.464). Hershkovitz suggested that the "near Tabatinga" series was made up of specimens from several localities; that the January, pale-bellied individuals (Fig. 10) may have originated from a single locality, 'perhaps' (1977, p. 464) on the southern bank of the Amazon, and that the saturate-bellied June–July specimens (Fig. 12) 'likely' (1977, p. 464) came from the opposite, north, Tabatinga bank.

According to Hershkovitz (1977), two of three specimens collected by C. Kalinowski in 1957 from Santa Cecília on the Río Manití, a small tributary south of the Río Amazonas just below Iquitos and above the mouth of the Río Napo in Peru, are similar to *niveiventris* (locality 35, Fig. 1). Of three specimens from the Río Yaquerana (locality 40, Fig. 1) and upper Javarí, one has a white ventrum like *niveiventris*, a second likewise, but with more grey on the undersurface of the limbs, and the ventrum of the third was dominantly buffy. Specimens from Apayacu, north of the Río Amazonas, below the mouth of the Río Napo (locality 36, Fig. 1), Intillama (close to San Pablo, Río Aguarico, locality 27), and Boca Río Curaray (locality 32) a right bank tributary of the Río Napo have underparts that conform to Lönnberg's *pygmaea*. Specimens from the Rio Copataza (locality 30) and Montalvo (locality 31) on the Río Bobonaza (localities in the Pastaza basin, north of the Río Marañón) are intermediate, with greyish and buffy underparts.

The specimens we examined in this study also conform with Hershkovitz' (1977) findings with respect to specimens found along both banks of the Solimões, i.e., there are some variability on the coloration of the underparts going from predominantly white to orange or grey irrespective of the river bank. In fact, our specimen CCM19,

391 collected in Benjamin Constant, right across the river from Tabatinga on the mouth of
392 the Javarí and on the south bank of the Solimões shows some orange pelage in its
393 underparts (Fig. 14a). Conversely, an individual we examined at the Museu Paraense
394 Emílio Goeldi in Belém, Brazil, collected in the Mamirauá Sustainable Development
395 Reserve on the left bank of the Solimões, opposite the town of Tefé, shows very white
396 pelage in its underparts (Fig. 14b) (unfortunately, no tissue from this Mamirauá
397 specimen was available for genetic analysis).
398 Regarding the Juruá specimens that Lönnberg (1940) found to be distinct from
399 *niveiventris*, believing they were typical *pygmaea*, Carvalho (1957) examined a
400 specimen from Seringal Oriente, on the right bank of the upper Rio Juruá in the state
401 of Acre, Brazil (locality 19, Fig. 1), and recorded that the color of the ventrum had a
402 thin, whitish median band on the underside, with the hairs on the ventrum changing to
403 yellowish and black towards the flanks; a pattern also found in the Olalla specimens
404 from João Pessoa (Eirunepé) (Fig. 7). Lönnberg (p.22) observed that "[O.] Thomas
405 has also recorded the typical species [*pygmaea*] as far west as Pebas, Peru." (locality
406 37, Fig. 1). Specimens NMNH 16610 from Sarayacu, Peru (locality 41, Fig. 1) and
407 NMNH 267506 from Huachi, Oriente, Río Pastaza, Ecuador (exact locality unknown,
408 but evidently somewhere in the region of localities 30 and 31, Fig. 1) are similar to
409 the João Pessoa (Eirunepé) specimens in Stockholm.
410 The phenotype typified in the Eirunepé specimens collected by A. M. Olalla,
411 evidently occurs, therefore, in other localities on the upper Juruá (Seringal Oriente
412 [locality 19] and Ocidente [locality18]), besides Pebas (Río Napo, Peru [37]),
413 Sarayacu (41) and the Río Pastaza, Ecuador. This group is distinct from specimens
414 north of the Solimões and perhaps Napo, and may be a distinct taxon, but although the

three Juruá specimens are grouped in the molecular phylogenetic analyses, their separation from other lineages south of the Solimões could be very recent.

4.3. Distribution and taxonomy of the pygmy marmoset

Cebuella occurs south of the Rio Japurá-Caquetá, in Colombia, extending north to the right bank of the Río Orteguaza, a left bank tributary of the upper Caquetá (Izawa, 1979; Defler, 2004). In Ecuador, it occurs throughout the eastern tropical Amazon lowlands, in the basins of the Marañón tributaries, the rios Tigre, Pastaza, Napo and Aguarico. In Peru, it occurs east from the Rio Santiago and the Río Cumbaza, and from the east bank of the Rio Huallaga, above the mouth of the Cumbaza, to the Ucayali valley, and the ríos Pachitea, Urubamba, and Manu of the upper reaches of the Madre de Dios (Aquino and Encarnación, 1994). In Bolivia, it has been recorded just south of the Rio Muyumanu (Buchanan-Smith et al., 2000), but it is otherwise believed to be restricted to the north of the Río Tahuamanu in the western Pando and the Rio Abunã along the northern border of Bolivia to the east. (Izawa, 1979; Izawa and Bejarano, 1981; Rylands et al., 1993; Buchanan-Smith et al., 2000; Porcel et al., 2010). In Brazil, it is restricted to the left banks of the rios Abunã and Madeira, and south of the rios Solimões-Amazonas and Japurá (Rylands et al., 1993; Bicca-Marques and Calegaro-Marques, 1995; Van Roosmalen and Van Roosmalen, 1997; Messias et al. 2011).

Defler (2004) and Van Roosmalen and Van Roosmalen (1997, 2016) indicated that *pygmaea* was the form occurring north of the rios Marañón and Amazonas-Solimões, east from the mouth of the Rio Japurá, south of the ríos Japurá-Caquetá and Caguán, and west as far as the Río Pastaza, and that *niveiventris* was the form south of

439 the Rio Amazonas-Solimões, west from the Rio Madeira to the lower Rio Huallaga
440 and the Rio Ucayali in Peru, and north of the Río Tahuamanu into Bolivia. Our
441 genetic analyses confirm the notion of two distinct taxa occupying these general range
442 limits with their lineages separating in the late Pliocene or early Pleistocene.
443 However, with the data currently available we cannot say *pygmaea* is a valid name for
444 animals found either in the north or in the south of the Solimões for all the reasons
445 already outlined above.
446 Unfortunately, in this study we had no tissue samples available for animals found
447 on the immediate vicinity of the left (north) bank of the Solimões and near Tabatinga.
448 Determining the phylogenetic relationship between these animals and our existing
449 samples would help shed light on the current uncertainties surrounding the taxonomy
450 of *Cebuella*.
451 One possibility we envisage, and that could complicate matters further, is that we
452 find that pygmy marmosets inhabiting forests adjacent to both banks of the Solimões
453 are genetically similar. This would support Hershkovitz' (1977) findings and our own
454 observations that there is great phenotypic variability and overlap in color morphs in
455 individuals collected in this area of Amazonia.
456 Such findings would appear surprising in light of what we know about Amazonian
457 biogeography and the role of large Amazonian rivers as important biogeographical
458 barriers to primates (Boubli et al., 2015). On the other hand, meandering, white-water
459 rivers such as the Solimões are known to be dynamic and to change their course often,
460 forming oxbow lakes and, in the process, transferring pieces of land from one side of
461 the river to the opposite bank (Ayres & Clutton-Brock, 1999, Gascon et al., 2000).
462 Animals that happen to be present on these pieces of land, in particular animals with

small home ranges such as pygmy marmosets, can be passively transferred from one bank of the river to the other. Such river dynamics would have favored intermittent contact between pygmy marmosets populations on opposite banks of the Solimões, potentially allowing them to interbreed. Only further sampling along the Solimões and further inland will help clarify this issue.

Conclusions

In this study, we generated phylogenetic hypotheses and calibrated time trees with very tight HPD intervals. This was possible due to the use of ddRADseq loci. Although ddRADseq and RADseq loci are generally considered more appropriate for population level questions than for phylogenetic reconstruction (Leaché et al., 2015), all principal types of genomic markers currently used in phylogenomic analyses are appropriate and informative for reconstructing phylogenetic relationships of groups that diverged during the Cenozoic (Collins and Hrbek, 2015; see also Eaton et al., (2017). The ddRADseq and RADseq loci are particularly suitable for more recent divergences since their information content peaks at 20 million years of divergence, and at even more recent divergence times their information content is greater than that of UCE or Exon loci (Collins and Hrbek, 2017). Thus it should not be surprising that in our study we were able to generate a robust phylogenetic hypothesis, since this hypothesis was based on 954 loci (with an average of 7.3–8.3x coverage) with 20588 variable sites of which 9628 were parsimony informative, assuming no more than 50% missing data which has empirically been shown to maximize phylogenetic informativeness and support (Streicher et al., 2016). ddRADseq data are easy to

generate (https://github.com/legalLab), and thus we hope primate phylogenomic analyses will become the norm in the near future.

The phylogenetic study strongly suggests that there are two distinct species of pygmy marmoset in the Brazilian Amazon, one in the Japurá basin and one south of the Solimões. The identification of further taxa will depend on a more complete and exhaustive molecular phylogenetic analysis, and corresponding analyses of phenotype variation throughout the vast range of the pygmy marmoset in Bolivia, Brazil, Colombia, Ecuador and Peru. Needed are genetic analyses and a thorough review of the pelage variation in specimens throughout the pygmy marmoset's broad distribution (Buchanan-Smith et al., 2000).

Gazetteer

Numbered localities are shown in Figure 1.

Bolivia

1. Ponton, Río Muyumanu, south bank, right bank tributary of the Río Tahuamanu, Pando, Bolivia, 11°31'S, 69°03'W. Field survey 1997. Buchanan-Smith et al. (2000).

Brazil

2. Japurá, Rio, right bank, middle, near Vila Bittencourt, Amazonas, Brazil, 1°50'32.9"S, 69°01'12"W. Specimens UFAM / CTGA-M720/M723/M724.
3. Tabatinga, vicinity of, town, north bank of the Rio Solimões, Amazonas, Brazil, 4°14'S, 69°56'W. Type locality of *Cebuella pygmaea* (Spix, 1823) in near this town, but not established as whether it is north of the Rio Solimões. Hershkovitz (1977; locality 171).
4. Javarí, Rio, mouth, south bank of the Rio Solimões, near Tabatinga, Amazonas, Brazil, 4°21'7.4", 70°2'18.4" (S4.352070, W70.038446). Type locality of *Cebuella pygmaea* (Spix), as restricted by Lönnberg (1940).
5. Benjamin Constant, Rio Solimões, right bank, Amazonas, Brazil, 4°19'48"S, 69°46'12". Specimen INPA 4041 [CCM19].
6. Pati, Rio, left bank, Jutaí-Solimões Ecological Station, Amazonas, Brazil, 3°18'36"S, 67°31'48"W. Specimen JT079.
7. Jutaí, Rio, Extractivist Reserve, Amazonas, Brazil. 3°13'12"S, 67°19'48"W. Specimen JT057.
8. Jutaí, Rio, Extractivist Reserve, left bank, Amazonas, Brazil. 3°44'24"S, 67°28'12"W (S3.74, W67.47). Specimen JT095.
9. Barroso, Comunidade, municipality of Uarini, Reserva de Desenvolvimento Sustentável Mamirauá, left bank of middle Rio Solimões, Amazonas, Brazil, 8°33'58"S, 72°47'W. Specimen RDSM323/MPEG37114.
10. Tefé (= Ega), Lago de, south bank of the Rio Solimões, Amazonas, Brazil, 3°27'S, 64°27'W, near sea level. Castelnau. Hershkovitz (1977; locality 182).

11. Ipixuna, Lago do, south of the Rio Solimões, Amazonas, Brazil, 3°52'S, 63°52'W. Hershkovitz (1977, locality 183). Type locality of *Cebuella pygmaea niveiventris* Lönnberg, 1940. Swedish Museum of Natural History. Allotype specimens NRM A59.4504.
12. Jacinto, Igarapé do, Rio Purus, right bank, 10 km south of Tapauá, Amazonas, Brazil, 5°42'S, 63°12'W. Specimen UFAM / CTGA-M170.
13. Bonfim, Rio Madeira, west (left) bank, opposite the town of Borba, Amazonas, Brazil, 4°20'S, 59°40'W. Sighting. M. G. M. Van Roosmalen and T. van Roosmalen, July, August 1996. Identified as *C. p. niveiventris*. Van Roosmalen and Van Roosmalen (1997).
14. Xadá, Lago, Rio Madeira, west (left) bank, Amazonas, Brazil, 5°15'36"S, 60°43'12"W. Specimen INPA / FR20 [INPA 5677].[Vencedor, Rio Madeira, west (left) bank, Amazonas, Brazil, 5°20'S, 60°45'W. Sighting. M. G. M. Van Roosmalen and T. van Roosmalen, July, August 1996. Identified as *C. p. niveiventris*. Van Roosmalen and Van Roosmalen (1997).]
15. Matupiri, Lago, Santa Maria, Rio Madeira, west (left) bank, Amazonas, Brazil, 5°33'15"S, 61°15'47"W. Sighting. M. G. M. Van Roosmalen and T. van Roosmalen, July, August 1996. Identified as *C. p. niveiventris*. Van Roosmalen and Van Roosmalen (1997). Specimen. Female CCM251.[Matupirizinho, Lago, Novo Jerusalem, Amazonas, Brazil, 5°33'28"S, 61°07'20"W. Sighting. M. G. M. Van Roosmalen and T. van Roosmalen, July, August,1996. Identified as *C. p. niveiventris*. Van Roosmalen and Van Roosmalen (1997).]

16. Democracia, Rio Madeira, west (left) bank, 15 km south of Manicoré, Amazonas, Brazil, 5°48'S, 61°26'W. Specimen INPA 4119 (CCM94). M. G. M. Van Roosmalen and T. van Roosmalen, July, August 1996. Identified as *C. p. niveiventris*.Van Roosmalen and Van Roosmalen (1997).
17. João Pessoa (= Eirunepé), left bank of the upper Rio Juruá, 6°50'30"S, 70°14'27"W, opposite its confluence with the Rio Tarauacá, Brazil (Paynter and Traylor, 1991). Collected by A. M. Olalla. 1936. Cited by Lönnberg (1940), who considered it to be *C. p. pygmaea*. Hershkovitz (1977; locality 176). Swedish Museum of Natural History. Specimens NRM: A61.2170, A61.2171, A61.2172, A61.2173, A61.2176, A61.2127, A61.3310.
18. Ocidente, Rio Juruá, right bank, Acre, Brazil, 8°33'58"S, 72°47'W (S8.566, W72.8). Specimens INPA / MNFS1019/1020/1361.
19. Seringal Oriente, right bank of upper Rio Juruá, Acre, Brazil, 8°48'S, 72°46'W, 100−200 m above sea level. Collected by F. C. Novaes and M. Moreira in 1951. Museu Paraense Emílio Goeldi (MPEG), Belém, Pará. Cited by Carvalho (1957) who indicated its similarity to the nominotypical *Cebuella pygmaea*, following Lönnberg (19430). Hershkovitz (1977; locality 89).
20. Santo Antônio, cachoeira, Rio Madeira, approximately 9 km upstream of Porto Velho, Rondônia, Brazil (08°46'46.4"S, 63°58'14.0"W). Specimen, Scientific Collection of the Federal University of Rondônia. UFROM 175, adult male, skull, skin and skeleton. Collected by Juliano Coragem and Ivonete Santa Rosa Gomes, October 10, 2009. Messias et al. (2011).

21. Antimari, Rio, west (left) bank tributary of the Rio Acre, Antimari State Forest (66,168 ha), traversed by the municipalities of Bujari, and Sena Madureira, Acre, Brazil, 9°01'15"−9°11'41"S, 68°00'19”−68°01'45”W, 250−300 m above sea level. Sighting. Calouro et al. (1991; see Bicca Marques and Calegaro-Marques, 1995).

22. Acre, Federal University of, Zoobotanical Park, urban district of Rio Branco, municipality of Rio Branco, Acre, Brazil. 9°56'30"−9°57'19"S, 67°52'08”−67°53'00”W, 155 m above sea level. Sighting. Bicca Marques and Calegaro-Marques (1995).

23. Rôla, Rio do, basin, municipalities of Rio Branco and Xapuri, 10°−11°S, 68°− 69°W, 216−260 m above sea level. Sighting. Brazil, IMAC (1993; see Marques and Calegaro-Marques, 1995).

43. Feijó, Acre, Brazil. Museu Paraense Emílio Goeldi, Belém, Pará, Brazil. 8°9'50"S, 70°21'14"W. MPEG 21854.44. Purus, Floresta Nacional, left bank of the Rio Pauini, Amazonas, Brazil. Approx. 7°42'S, 67°06'W. Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas. INPA 7256 (field #RS44).

Colombia

24. Letícia, Rio Solimões, Colombia, 4°15'S, 69°56'W, 100 m above sea level. P. Hershkovitz, June 1952. Hershkovitz (1977; locality 58e).

25. Puerto Limón, municipality of San Miguel de Mocoa, department of Putumayo, Colombia, 1°01'57.6"S, 76°26'42.2"W. Sighting. Izawa (1979).

602

603 **Ecuador**

604

605 26. La Coca, Río Napo, Ecuador, 0°28'S, 76°58'W, 258 m above sea level.
606 Jiménez de la Espada, 1865. Hershkovitz (1977; locality 63). [San Pablo,
607 Río Aguarico, right bank, Ecuador, 0°16'27"S, 76°25'29"W. Field research
608 and genetic study 2001−2003. Yépez et al. (2005) and Monteros et al.
609 (2011).]
610 27. Intillama (Indillama), Río Napo, left bank, Ecuador, 0°27'S, 76°31'W, 250 m
611 above sea level. P. Hershkovitz, June 1936. Hershkovitz (1977; locality 65).
612 [Sacha, Río Napo, north bank, 30 km south of San Pablo (Río Aguarico),
613 Ecuador, 0°28'49.5"S, 76°27'43.4"W (UTM, 18 Zone Datum PSA 56
614 337296E, 9946861N). Field research 2001−2003. Yépez et al. (2005).]
615 28. Zancudo Cocha, Laguna, Río Aguarico, right bank, approximately 150 km
616 west of San Pablo (Río Aguarico), border of Cuyabeno National Park,
617 Ecuador, 0°35'56.9"S, 75°29'24.6"W (UTM, 18 Zone Datum PSA 56
618 445459E, 9933749N. Field research 1997. Yépez et al. (2005).
619 29. Amazoonico, Río Arajuno, south bank, approximately 150 km west of Sacha
620 (Río Napo) and San Pablo (Río Aguarico), 1°03'1.8"S, 77°31'20.2"W
621 (UTM, 18 Zone Datum PSA 56 219290E, 9883728N). Field research 2003.
622 Yépez et al. (2005). [Yasuní National Park, at km 47 of the Pompeya Sur-Iro
623 road to Maxus Ecuador, Inc., southeastern Ecuador, 0°42'01"S, 76°28'05"W.
624 Field research 1995. Youlatos (1999).]

30. Copataza, Río, Río Pastaza, Ecuador, 2°07'S, 77°27'W, 450 m above sea level. R. Olalla, April 1939, C. Buckley, 1877-1878. Hershkovitz (1977, locality 74).
31. Montalvo, Río Bobonaza, Río Pastaza, Ecuador, 2°04'S, 76°58'W, 314 m above sea level. R. Olalla, February 1932. Hershkovitz (1977; locality 75a).

Peru

32. Río Curaray, mouth, right bank tributary of the Río Napo, Peru, 2°22'S, 74°05'W, 140 m above sea level. Olalla Bros. October-December 1925, March, 1926. Hershkovitz (1977, locality 80).
33. Mishana, Río Nanay, left bank tributary of the Rio Amazonas, Peru, 3°45'S, 73°35'W. Field research 1974. Kinzey *et al.* (1975). Hershkovitz (1977; locality 94b).
34. Iquitos, Río Amazonas (or Marañón), left bank, Peru, 3°44'S, 73°15'W, 106 m above sea level. Hershkovitz (1977; locality 92).
35. Santa Cecília, Rio Manití, a small tributary south of the Río Amazonas just below Iquitos and above the mouth of the Río Napo, Peru, 3°26'S, 72°46'W, 110 m above sea level. C. Kalinowski, January 1957. Hershkovitz (1977; locality 89).
36. Apayacu, Río, north of the Rio Amazonas, Peru, 3°21'S, 72°07'W, about 100 m above sea level. Hershkovitz (1977; locality 86).
37. Pebas, Río Marañón, Peru, 3°10'S, 71°48'W, 100 m above sea level. R. W. Hendee, January-February 1928. Hershkovitz (1977; locality 85).

38. Chimbote, Río Marañón, Peru, 3°49'S, 70°41'W. Locality of Cruz Lima (1945). Hershkovitz (1977; locality 83).
39. Tapiche, Río, right bank, just below mouth of its tributary, the Rio Blanco, Loreto, Peru, 5°35'S, 73°53'W. Field research. Soini (1987).
40. Yaquerana, Río, mouth, left bank, Peru, 5°43'S, 72°58'W. C. Kalinowski, September 1957. Hershkovitz (1977; locality 118).
41. Sarayacu, Río Ucayali, Peru, 6°44'S, 75°07'W. Olalla Bros. July, March April 1927. Castelnau and Deville, 1847, presumably on right bank. Hershkovitz (1977; locality 102).
42. Manu National Park, Cocha Cashu Biological Station, Rio Madre de Dios, Peru, 11°54'S, 71°22'W, elevation about 400 m). Field research. Terborgh (1983).

Acknowledgements

Our most sincere thanks to the Department of Zoology of the Swedish Museum of Natural History, Stockholm, in particular Daniela C. Kalthoff, Mammal Curator, and Sandra Gytare for supplying the images of the allotype of *Cebuella pygmaea niveiventris* and the Eirunepé, Juruá specimens. Also to Darrin P. Lunde, Collection Manager, Mammal Department of the National Museum of Natural History, Smithsonian Institution, Washington, DC, who most kindly allowed ABR to examine the museum's *Cebuella* specimens. Molecular analyses and six field expeditions were funded in part by grants from CNPq/SISBIOTA-BioPHAM (grant no. CNPq 563348/2010) to IPF, CAPES/PRO-AMAZONIA/AUXPE (grant no. 3261/2013) to

673 IPF and HS, and NSF/FAPESP “Dimensions of Biodiversity” (grant nos. NSF
674 1241066 and FAPESP 12/50260-6) to Joel Cracraft and Lucia Lohmann. Permission
675 to conduct fieldwork and to collect tissue samples was granted by IBAMA (License
676 N° 005/2005 – CGFAU/LIC) and ICMBio.
677
678
679 **References**
680
681 Aberer, A.J., Kobert, K., Stamatakis, A., 2014. ExaBayes: massively parallel
682 Bayesian tree inference for the whole-genome era. Mol. Biol. Evol. 31, 1−8. DOI:
683 10.1093/molbev/msu236.
684
685 Aquino, R, Encarnación, F., 1994. Primates of Peru / Los Primates del Perú. Prim.
686 Rep. 40, 1−130.
687
688 Ayres, J. M. and Clutton-Brock, T. H., 1992. River Boundaries and Species Range
689 Size in Amazonian Primates. Am. Nat. 140, 531-537.
690
691 Bicca-Marques, J.C., Calegaro-Marques, C., 1995. Updating the known distribution
692 of the pygmy marmoset (*Cebuella pygmaea*) in the state of Acre, Brazil. Neotrop.
693 Primates 3, 48−49.
694
695 Boubli, J.P., Ribas, C., Lynch, J. W., Alfaro, M. E., Nazareth, M., Silva, F., … Farias,
696 I.P., 2015. Spatial and temporal patterns of diversification on the Amazon : A test

of the riverine hypothesis for all diurnal primates of Rio Negro and Rio Branco in Brazil. Mol. Phylogenet. Evol., 82, 400–412. http://doi.org/10.1016/j.ympev.2014.09.005

Brazil, IMAC., 1993. Relatório de Caracterização Preliminar da Bacia Hidrográfica do Riozinho do Rolâ. Report, Instituto de Meio Ambiente do Acre (IMAC), Rio Branco, Acre.

Buchanan-Smith, H.M., Hardie, S.M., Caceres, C., Prescott, M.J., 2000. Distribution and forest utilization of *Saguinus* and other Primates of the Pando Department, northern Bolivia. Int. J. Primatol. 21, 353−379.

Buckner, J.C., Lynch Alfaro, J., Rylands, A.B., Alfaro, M.E., 2015. Biogeography of the marmosets and tamarins (Callitrichidae*).* Mol. Phylogenet. Evol. 82(B), 413−425.

Cabrera, A., 1957. Catalogo de los mamíferos de América del Sur. Rev. Mus. Argentino de Cienc. Nat. “Bernardino Rivadavia” 4, 1−307.

Carvalho, A. 1957. Alguns Mamíferos do Acre Ocidental. Boletim do Museu Paraense Emílio Goeldi 6, 1-9.

Collins, R.A., Hrbek, T. 2017. An in silico comparison of protocols for dated phylogenomics. Systematic Biology, in press

720 Coyne, K.J., Burkholder, J.M., Feldman, R.A., Hutchins, D.A., Cary, S.C., 2004.
721 Modified serial analysis of gene expression method for construction of gene
722 expression profiles of microbial eukaryotic species. Appl. Environ. Microbiol. 70,
723 5298-5304. DOI: 10.1128/AEM.70.9.5298
724
725 Cruz Lima, E. da., 1945. Mammals of Amazônia, Vol. 1. General Introduction and
726 Primates. Contribuições do Museu Paraense Emílio Goeldi de História Natural e
727 Etnografia, Belém do Pará.
728
729 Defler, T. R. 2004. Conservation International Tropical Field Guide Series: Primates
730 of Colombia. Conservation International, Bogotá, Colombia, 550 pp.
731
732 Drummond, A.J., Suchard, M.A., Xie D., Rambaut, A., 2012. Bayesian phylogenetics
733 with BEAUti and the BEAST 1.7 Mol. Biol. Evol. 29, 1969−1973.
734
735 Eaton, D.A., 2014. PyRAD: assembly of de novo RADseq loci for phylogenetic
736 analyses. Bioinformatics. 30, 1844–9
737
738 Eaton, D.A., Spriggs, E.L., Park, B., Donoghue, M.J., 2017. Misconceptions on
739 missing data in RAD-seq phylogenetics with a deep-scale example from
740 flowering plants. Systematic Biology 66, 399–412. doi: 10.1093/sysbio/syw092
741
742 Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and
743 high throughput. Nucleic Acids Res. 32, 1792−1797

744

745 Elliot, D.G., 1913. A Review of the Primates. 3 vols. American Museum of Natural
746 History, New York.
747

748 Gascon, C., Malcolm, J. R., Patton, J. L., da Silva, M. N. F., Bogart, J. P., Lougheed,
749 S. C., … Boag, P. T., 2000. Riverine barriers and the geographic distribution of
750 Amazonian species. PNAS 97, 13672–13677.
751 http://doi.org/10.1073/pnas.230136397
752

753 Groves, C. P., 1989. A Theory of Human and Primate Evolution. Oxford Science
754 Publication, Oxford.
755

756 Groves, C. P., 2001. Primate Taxonomy. Smithsonian Institution Press, Washington,
757 DC.
758

759 Groves, C. P., 2005. Order Primates. In: Wilson, D.E., Reeder D. M. (eds), Mammal
760 Species of the World. A Taxonomic and Geographic Reference. Third edition.
761 Johns Hopkins University Press, Baltimore, MD, pp. 111–184.
762

763 Hershkovitz, P., 1977. Living New World Monkeys (Platyrrhini) with an Introduction
764 to Primates, Vol. 1. The Chicago University Press, Chicago, IL.
765

766 Hill, W.C.O., 1957., Primates. Comparative Anatomy and Taxonomy III. Pithecoidea
767 Platyrrhini (Families Hapalidae and Callimiconidae). Edinburgh University Press,
768 Edinburgh.
769
770 Izawa, K., 1979. Studies on peculiar distributio pattern of *Callimico*. Kyoto
771 University Overseas Research. Reports of NewWorld Monkeys, 1−19.
772
773 Izawa, K., Bejarano, G., 1981. Distribution ranges and patterns of nonhuman primates
774 in western Pando, Bolivia. Kyoto University Overseas Research. Reports of
775 NewWorld Monkeys, 1−12.
776
777 Kinzey, W.G., Rosenberger A.L., Ramirez, M., 1975. Vertical clinging and leaping in
778 a Neotropical anthropoid. Nature 255, 327−328.
779
780 Leaché, A.D., Chavez, A.S., Jones, L.N., Grummer, J.A., Gottscho, A.D., Linkem,
781 C.W., 2015. Phylogenomics of phrynosomatid lizards: Conflicting signals from
782 sequence capture versus restriction site associated DNA sequencing. Genome
783 Biology and Evolution 7, 706–719. doi: 10.1093/gbe/evv026
784
785 Lönnberg, E., 1940. Notes on marmosets. Ark. Zool., Stockholm 32A(10), 1−22.
786
787 Marivaux, L., Adnet, L., Altamirano-Sierra, A.J., Pujos, F., Ramdarshan, A., Salas-
788 Gismondi, R., Tejada-Lara, J.V., Antoie, P.-O., 2016. Dental remains of cebid
789 platyrrhines from the earliest late Miocene of western Amazonia, Peru:

790 macroevolutionary implications on the extant capuchin and marmoset lineages.
791 Am. J. Phys. Anthropol. 161, 478-493. DOI 10.1002/ajpa.23052.
792
793 Messias, M.R., Coragem, J.T., Gomes, I.S.R., Oliveira, M.A., Bonavigo, P. H.,
794 Nienow, S. dos S., Souza, E.S. de., 2011. Southern extension of the geographical
795 range of the pygmy marmoset *Cebuella pygmaea niveiventris* (Lönnberg, 1940)
796 in the southwestern Amazon basin, state of Rondônia, Brazil. Neotrop. Primates
797 18, 30−31.
798
799 Napier, J.R. and Napier, P.H., 1967. A Handbook of Living Primates. Academic
800 Press, London.
801
802 Napier, P.H., 1976. Catalogue of Primates in the British Museum (Natural History).
803 Part 1: Families Callitrichidae and Cebidae. British Museum (Natural History),
804 London.
805
806 Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and
807 evolution in R language. Bioinformatics 20, 289−290. DOI:
808 10.1093/bioinformatics/btg412
809
810 Paynter Jr., R.A. and Traylor Jr., M.A., 1991. Ornithological Gazetteer of Brazil.
811 A−M. President and Fellows of Harvard College Cambridge, MT.
812

813 Perelman P., Johnson, W.E., Roos, C., Seuánez, H.N., Horvath, J.E., Moreira, M.A.,
814 Kessing, B., Pontius, J., Roelke, M., Rumpler, Y., Schneider, M.P.C., Silva, A.,
815 O'Brien, S.J., Pecon-Slattery, J., 2011. A molecular phylogeny of living primates.
816 PLoS Genet. 7, e1001342.
817
818 Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012., Double
819 digest RADseq: an inexpensive method for de novo SNP discovery and
820 genotyping in model and non-model species. PLoS One 7, e37135. DOI:
821 10.1371/journal.pone.0037135
822
823 Ramirez, M., Freese, C.H., Revilla, J., 1977. Feeding ecology of the pygmy
824 marmoset, *Cebuella pygmaea*, in northeastern Peru. In: Kleiman, D.G. (ed.), The
825 Biology and Conservation of the Callitrichidae. Smithsonian Institution Press,
826 Washington, DC, pp.91−104.
827
828 Rylands, A.B., Coimbra-Filho, A.F., Mittermeier, R.A., 1993. Systematics,
829 distributions and some notes on the conservation status of the Callitrichidae. In:
830 Rylands, A.B. (ed.), Marmosets and Tamarins: Systematics, Behaviour, and
831 Ecology, Oxford University Press, Oxford, pp.11−77.
832
833 Rylands, A.B., Coimbra-Filho, A.F., Mittermeier, R.A. 2009. The systematics and
834 distributions of the marmosets (*Callithrix, Callibella, Cebuella*, and *Mico*) and
835 callimico (*Callimico*) (Callitrichidae, Primates). In: Ford, S. M. L. Porter, L.,

836 Davis, L. C. (eds.), The Smallest Anthropoids: The Marmoset/Callimico
837 Radiation, Springer, New York, pp.25–61.
838
839 Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory
840 Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
841
842 Soini, P., 1982. Ecology and population dynamics of the pygmy marmoset, *Cebuella*
843 *pygmaea*. Folia Primatol. 39, 1−21.
844
845 Soini, P., 1993. The ecology of the pygmy marmoset, *Cebuella pygmaea*: some
846 comparisons with two sympatric tamarins. In: Rylands, A.B. (Ed.), *Marmosets*
847 *and Tamarins: Systematics, Behaviour and Ecology*. Oxford University Press,
848 Oxford, pp.257−261.
849
850 Soini, P., 1987. Sociosexual behavior of a free-ranging *Cebuella pygmaea*
851 (Callitrichidae, Platyrrhini) troop during postpartum estrus of its reproductive
852 female. *Am. J. Primatol.* 13, 223−230.
853
854 Spix, J.B. von, 1823. Simiarum et Verspertilionum Brasiliensium Species Novae. F.S.
855 Hübschmann, Munich.
856
857 Spix, J. B. von and Martius. C. F. von, 1938. Viagem pelo Brasil. Translation of Spix,
858 J. B, von and Martius. C. F. von, 1831. Reise in Brasilien auf Behehl Sr. Majestat
859 Maximilian Joseph I., Königs von Baiern, in den Jahren 1817 bis 1820. Volume

3. M. Lindauer, Munich. Translation by L.F. Lahmeyer. Instituto Histórico e Geográfico Brasileiro, Imprensa Nacional, Rio de Janeiro.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312−1313. DOI: 10.1093/bioinformatics/btu033

Streicher, J.W., Schulte, II J.A., Wiens, J.J., 2016. How should genes and taxa be sampled for phylogenomic analyses with missing data? An empirical study in iguanian lizards. Systematic Biology 65, 128–145. doi: 10.1093/sysbio/syv058

Van Roosmalen, M.G.M., Van Roosmalen, T., 1997. An eastern extension of the geographical range of the pygmy marmoset, *Cebuella pygmaea*. Neotrop. Primates 5, 3−6.

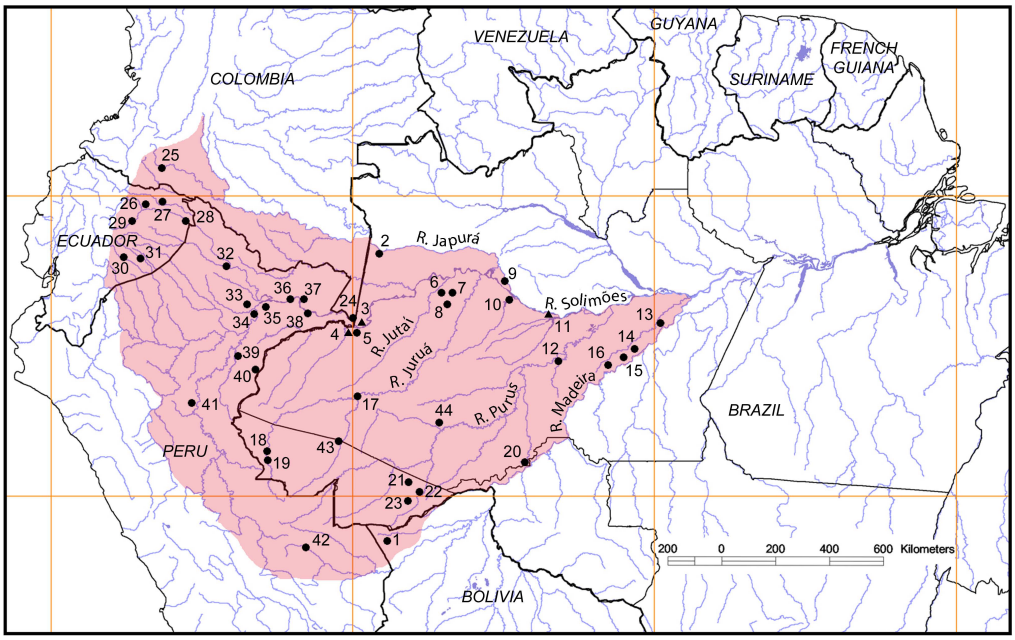
Van Roosmalen, M.G.M, Van Roosmalen, T., 2016. On the origin of allopatric primate species. Biodiv. J. 7, 117−198.

Vieira, C. da C., 1955. Lista remissiva dos mamíferos do Brasil. Arq. Zool., São Paulo 8(11), 341−374.

Werle, E., Schneider, C., Renner, M., Völker, M., Fiehn, W., 1994. Convenient single-step, one tube purification of PCR products for direct sequencing. Nucleic Acids Res. 22, 4354−4355.

884 **FIGURES**

885



886

887 **Fig. 1**. Distribution of the pygmy marmoset (*Cebuella*). Numbers correspond to the
888 gazetteer. The black triangles are type localities. Localities of the samples used in the
889 molecular analysis of this study: **2.** Japurá (CTGA-M720, 723, 724); **5.** Benjamin
890 Constant, upper Rio Solimões (CCM19); **6.** Rio Jutai, Rio Patiá, (JT079, JT095); **8.**
891 Rio Jutai, Extractivist Reserve (JT057); **12.** Jacinto, Rio Purus (CTGA-M170); **14.**
892 Lago Xada, Rio Madeira (FR 20); **18.** Ocidente, upper Rio Juruá (MNFS 1019, 1020,
893 1361).

894

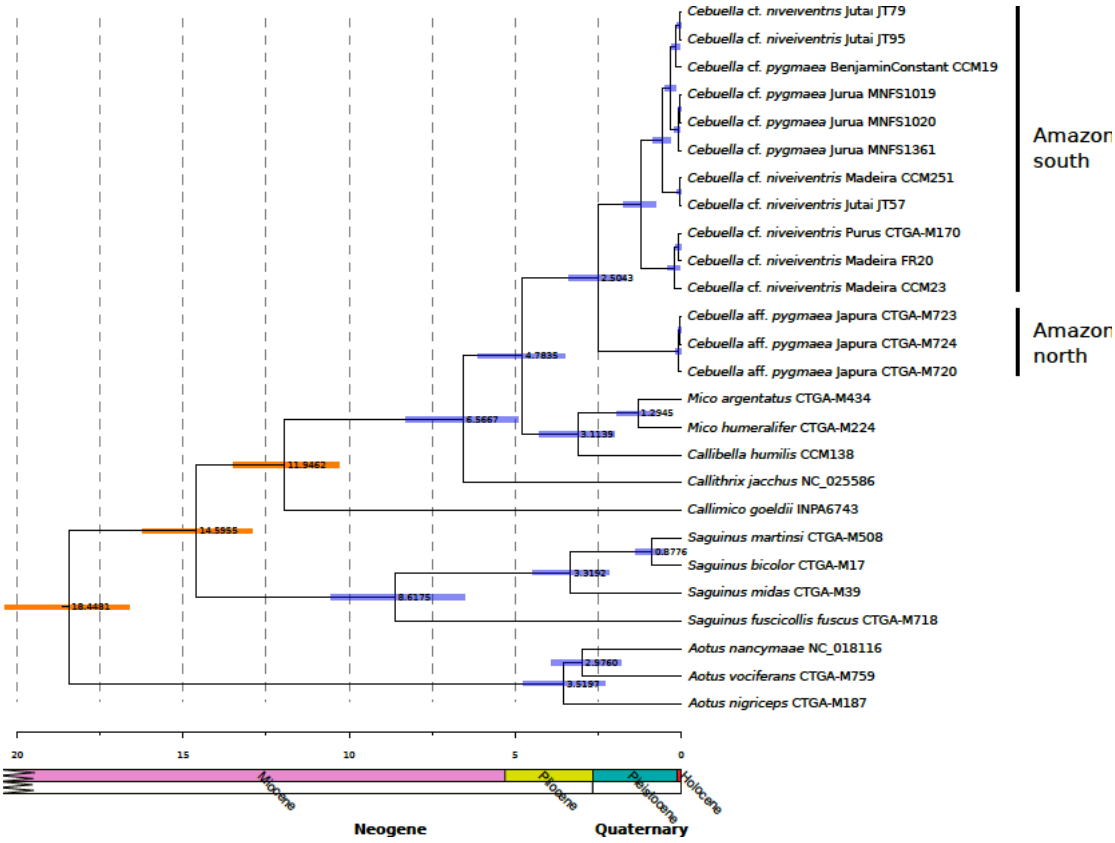


Fig. 2. BEAST cytochrome *b* time tree for 26 primate samples, including 14 pygmy marmosets - including 3 *Cebuella* aff. *pygmaea* (CTGA-M 720, 723, 724), 4 *Cebuella* cf. *pygmaea* (MNFS 1019, 1020, 1361; CCM 19) and 7 *Cebuella* cf. *niveiventris* (JT 57, 95, 79; CTGA-M170; FR 20; CCM 251, 23) – and 12 other taxa for comparative purposes. Numbers in nodes correspond to time in millions of years and error bars represent 95% HPD intervals. Divergences represented by orange error bars were used as calibration points. Bayesian posterior probabilities are all equal to 1, except for the sister taxon relationship between sample MNFS 1019 and 1020 supported by 0.95, sample FR 20 and CTGA-M170 supported by 0.94, sample CTGA-M724 and 723 supported by 0.55, and in the outgroups *Aotus nancymaae* was sister taxon of *Aotus vociferans* with pp = 0.77. See supplementary materials, Figure 1, for a map showing the localities for all specimens used in this phylogenetic analysis

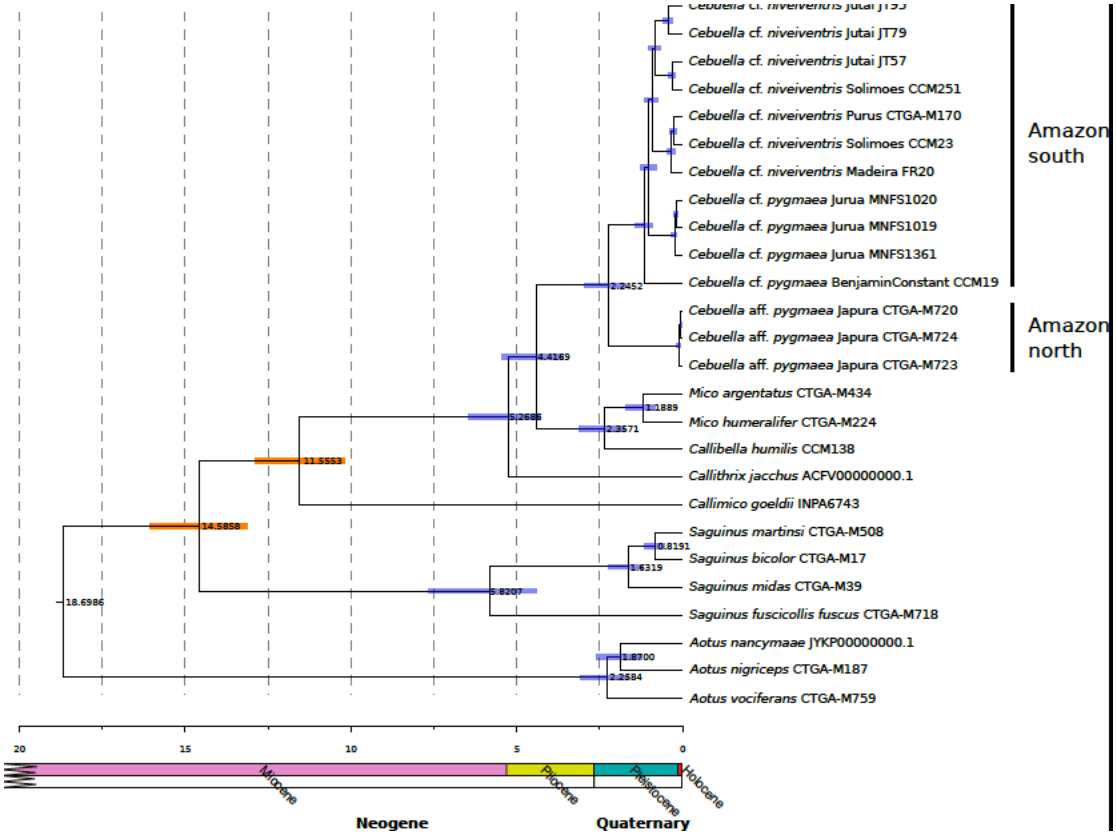


Fig. 3. BEAST ddRAD time tree for the same taxa as in Figure 3. Numbers at nodes correspond to time in millions of years and error bars represent 95% HPD intervals. Divergences represented by orange error bars were used as calibration points. Bayesian posterior probabilities are all equal to 1, except for the sister taxon relationship between sample MNFS 1019 and 1020 supported by 0.87 and sample CCM 23 and CTGA-M170 supported by 0.76. See supplementary materials, Figure 1, for a map showing all localities for the specimens used in this phylogenetic analysis.

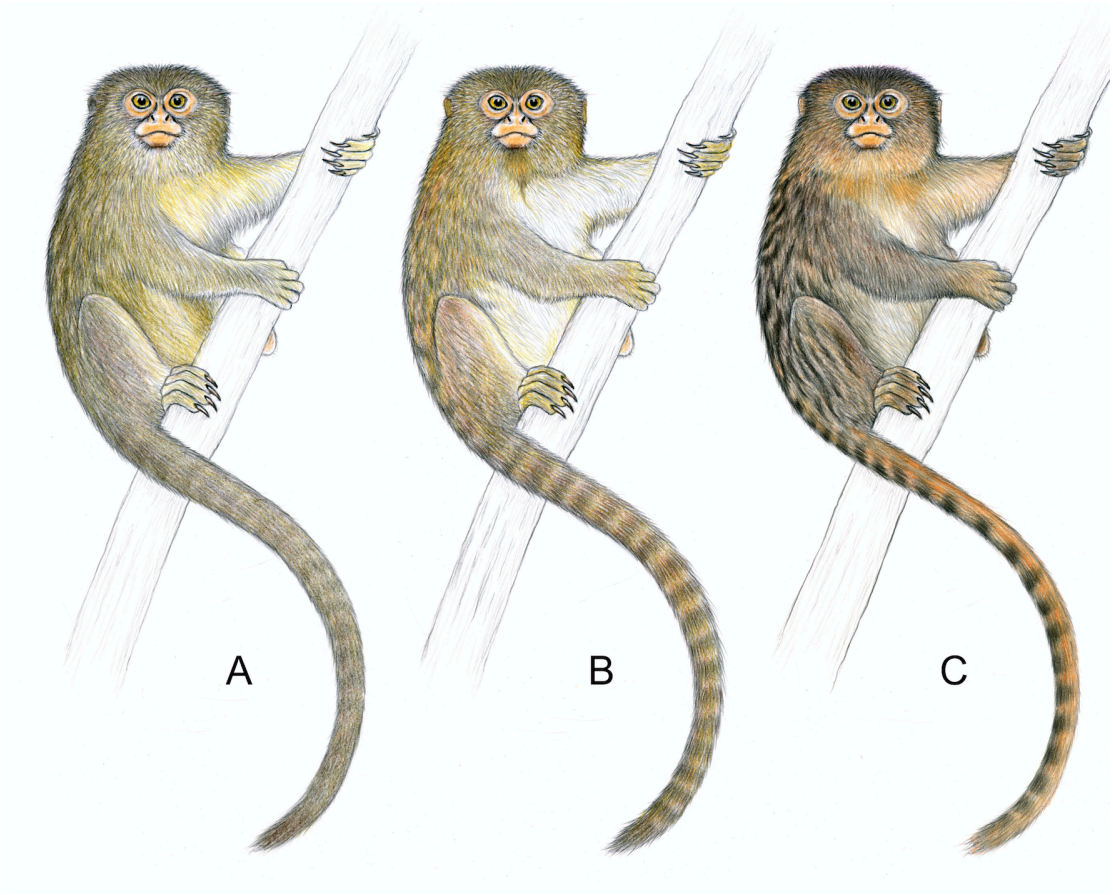


Fig. 4. From left to right, A) *Cebuella* aff. *pygmaea* from the upper Rio Japurá, Brazil (based on specimens from locality 2, Figure 1), B) *Cebuella* cf. *niveiventris* from the south of the Solimões (based on specimens from localities 6, 7, 8, 12, 14, 15, 16 and 44 in Figure 1), C) *Cebuella* cf. *pygmaea* from the upper Rio Juruá (based on specimens from locality 18, Figure 1). Illustrations copyright Stephen D. Nash/Conservation International.

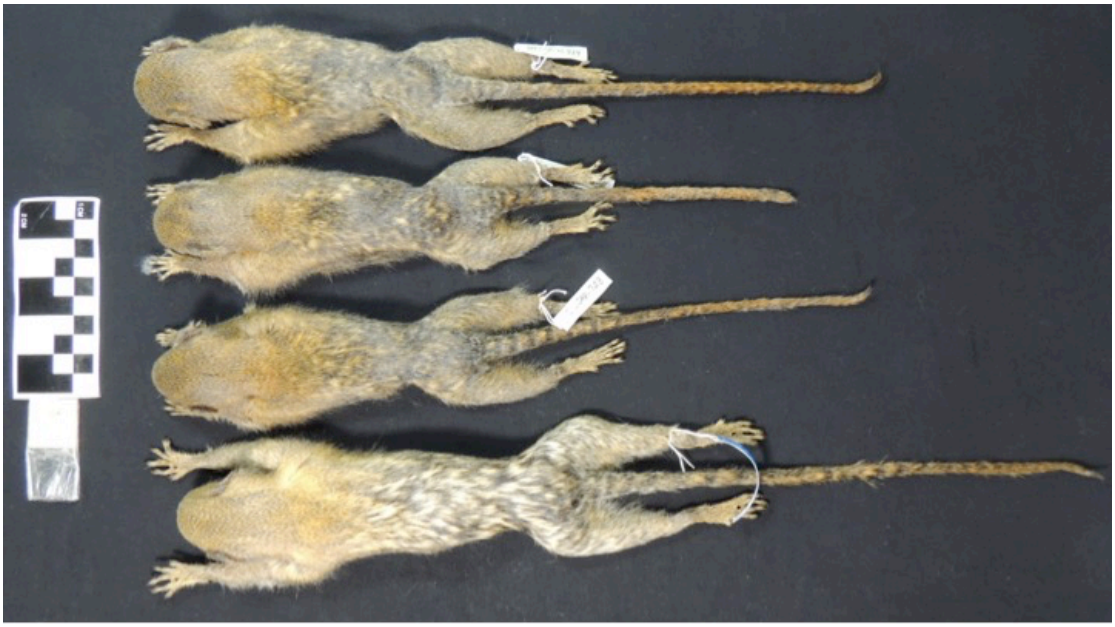


Fig. 5. A) Dorsal and B) ventral views of pygmy marmosets. Largest individual is *Cebuella* cf. *niveiventris* from the Rio Purus (CTGA-M170), locality 12 in Figure 1. Three small individuals are from the middle Rio Japurá, Brazil (CTGA-M720, 723 and 724), locality 2 in Figure 1. Photo by Jean P Boubli.

Fig. 6. The mounted type specimen of *Cebuella niveiventris* Lönnberg, Department of Zoology, Swedish Museum of Natural History, Stockholm. Specimen A61.330, Lago do Ipixuna, south of the Rio Solimões, Amazonas, Brazil, 3°52'S, 63°52'W. Locality 11 in Figure 1. Photo courtesy of the Department of Zoology, Swedish Museum of Natural History, Stockholm.

Fig. 7. Ventral view of specimen NRM 61.2127. Swedish Museum of Natural History, Stockholm. João Pessoa (= Eirunepé), left bank of the upper Rio Juruá, 6°50'30"S, 70°14'27"W, opposite its confluence with the Rio Tarauacá, Brazil.

939 Locality 17 in Figure 1. Photo courtesy of the Zoology Department of the Swedish
940 Museum of Natural History, Stockholm.
941
942 **Figure 8**. Mounted type specimen, *Cebuella pygmaea* Spix, Zoologische
943 Staatssammlung München Museum. Vicinity of Tabatinga, north bank of Rio
944 Solimões, western Amazonas at Colombian Frontier, Brazil. Locality 3 in Figure 1.
945 (http://www.zsm.mwn.de/mam/i/Jacchus_pygmaeus_20_D.jpg)
946
947 **Figure 9.** Ventral view of specimens UFAM / CTGA-M720/M723/724, Japurá, Rio,
948 right bank, middle, near Vila Bitencourt, Amazonas, Brazil, 1°50'32.9"S,
949 69°01'12"W. Locality 2 in Figure 1. Photo by Ingrid Macedo
950
951 **Figure 10.** Ventral view of specimens NMNH 336309 and 336312 from near
952 Tabatinga, Amazonas (Locality Captured in January 1963 on behalf of the NIH
953 California National Primate Research Center, San Diego, California, and sent to the
954 NMNH in February, March and April 1963. National Museum of Natural History,
955 Smithsonian Institution, Washington DC. Photo by Anthony B. Rylands.
956
957 **Figure 11.** Ventral view of specimen NMNH 336302, female, from near Tabatinga,
958 Amazonas. Captured in January 1963 on behalf of the NIH California National
959 Primate Research Center, San Diego, California. National Museum of Natural
960 History, Smithsonian Institution, Washington, DC. Photo by Anthony B. Rylands.
961

Figure 12. Ventral view of specimen NMNH 336304, female, from near Tabatinga, Amazonas. Captured in January 1963 on behalf of the NIH California National Primate Research Center, San Diego, California. National Museum of Natural History, Smithsonian Institution, Washington, DC. Photo by Anthony B. Rylands.

Figure 13. Ventral views of specimens NMNH 337322, 337325, 337323, 337329, from near Tabatinga, Amazonas. Captured in August 1963 on behalf of the NIH California National Primate Research Center, San Diego, California. National Museum of Natural History, Smithsonian Institution, Washington, DC. Photo by Anthony B. Rylands.

Figure 14. A) Specimen CCM19 of *C.* cf. *pygmaea* from Benjamin Constant, Brazil **B)** Specimen MPEG 37114 of *C.* cf. *pygmaea* from the Reserva do Desenvolvimento Sustentável, Mamirauá, Brazil.

TABLES

Table 1

List of voucher specimens and tissue samples used in this study and their localities.

Museum #	**Original #**	**Institution / Tissue #**	**Species**	**Locality**
RDSM no#	JT057	JT057	*Cebuella p. pygmaea*	Brazil, Amazonas: RESEX do
RDSM no#	JT079	JT079	*Cebuella p. pygmaea*	Brazil, Amazonas: ESEC Juta
RDSM no#	JT095	JT095	*Cebuella p. pygmaea*	Brazil, Amazonas: RESEX do
MPEG22954	MNFS1019	INPA / MNFS1019	*Cebuella p. pygmaea*	Brazil, Acre: Ocidente, right b
MPEG22953	MNFS1020	INPA / MNFS1020	*Cebuella p. pygmaea*	Brazil, Acre: Ocidente, right b
MPEG22952	MNFS1361	INPA / MNFS1361	*Cebuella p. pygmaea*	Brazil, Acre: Ocidente, righ b
INPA4041	MvR30=CCM19	INPA / MvR30=CCM19	*Cebuella p. pygmaea*	Brazil, Amazonas: Benjamin
INPA5677	FR20	INPA / FR20	*Cebuella p. niveiventris*	Brazil, Amazonas; left bank R

INPA7250	CCM23	INPA / CCM23	*Cebuella p. niveiventris*	Unknown locality
INPA7252	SISPUR-M170	UFAM / CTGA-M170	*Cebuella p. niveiventris*	Brazil, Amazonas: Igarapé do
INPA7254	SISJAP-M723	UFAM / CTGA-M723	*Cebuella nv sp*	Brazil, Amazonas: right bank
INPA7253	SISJAP-M720	UFAM / CTGA-M720	*Cebuella nv sp*	Brazil, Amazonas: right bank
		Genbank	*Aotus nancymaae*	Not available
INPA no#	SISPUR-M187	UFAM / CTGA-M187	*Aotus nigriceps*	Brazil, Amazonas, Jacinto, ric
INPA no#	SISJAP-M759	UFAM / CTGA-M759	*Aotus vociferans*	Brazil, Amazonas, Taboca, ric
INPA4090	CCM 138	INPA / CCM 138	*Callibella humilis*	Brazil, Mato Grosso, Aripuan
RDSM no#	HGLN 02	INPA / HGLN 02	*Callibella humilis*	Aripuana river
INPA6743	RS 34	INPA / RS 34	*Callimico goeldii*	Not available
		Genbank	*Callithrix jacchus*	Not available
INPA no#	SISTAP-M364	UFAM / CTGA-M434	*Mico argentata*	Brazil, Pará, Itapuama, Tapaj
INPA no#	SISTAP-M224	UFAM / CTGA-M224	*Mico humeralifer*	Brazil, Pará, Cameta, Tapajos
RDSM no#	FES 09	INPA / FES 09	*Mico marcai*	Brazil, Mato Grosso, Aripuan
UFAM no#	SISIS 17	UFAM / CTGA-M17	*Saguinus bicolor*	Brazil, Amazonas, Manaus
INPA no#	SISTRO-M508	UFAM / CTGA-M508	*Saguinus martinsi*	Brazil, Pará, Saracá-Taquera,
INPA no#	SISJAT-M39	UFAM / CTGA-M39	*Saguinus midas*	Brazil, Amazonas, Jatapu

Table 2

Morphological comparisons of the underside pelage coloration of *Cebuella* specimens in the Brazilian Amazon: 1) south of the Rio Solimões, 2) Eirunepé-Acre, upper Juruá basin, southwestern Brazilian Amazon

	South Solimoes	**Juruá Eirunepé/Acre**
General ventral appearance	Light-colored, buff to whitish undersides; throat and chest ochraceous orange, sometimes throat is buffy to whitish in the center; the ochraceous orange throat and upper chest contrast with a pure white chest; undersides may be more greyish towards the abdomen (specially laterally) and legs	Dark-colored (dull yellowish brown) undersides; dull ochraceous orange t brownish throat, sometimes throat is whitish in the center; the ochraceous brownish coloration is limited mostly the base of the neck, slightly contrast the whitish to yellowish brown chest aspect of chest varies from white to l tawny (yellowish-brown); yellowish black hairs may cover the entire abd penetrate the abdominal region form lateral line that marginate a whitish or wide median band along the entir underside
Throat	ochraceous orange, sometimes buffy to whitish in the center;	dull ochraceous orange to brownish, sometimes buffy to whitish in the cer

Chest	ochraceous orange upper chest contrast with a whitish chest and belly	varies from white to buff to tawny (y brown)
Belly	buff to whitish, may be more greyish towards the abdomen (specially laterally) and legs	yellowish and black hairs may cover entire abdomen or penetrate the abd region forming a lateral line that mar whitish narrow or wide median banc
Limbs	inner surface of arms and legs varying from modified agouti to dominantly yellowish or grayish, general coloration of tights similar to belly	inner surface of arms dominantly yel and of legs modified agouti to entirel or whitish
Tail	tail modified agouti with upper surface barred black, under surface brownish with banding shadowy; dorsal darker bands 20+ in number	tail modified agouti with upper surfa black, under surface evenly ochracec general aspect of no banding

986

987 **SUPPLEMMENTARY MATERIALS**

988

989 **Figure 1.** Localities for the pygmy marmoset specimens used in our phylogenetic
990 analysis, numbered as in the gazetteer in the main text. 2 – CTGA M720, 723 and
991 724, right bank the Rio Japurá; 5 – CCM19, Benjamin Constant; 6 – JT059, ESEC
992 Jutaí-Solimões, left bank of the Rio Jutaí; 7 – JT057, RESEX do Rio Jutaí, right bank
993 of the Rio Jutaí; 8 – JT095, RESEX do Rio Jutaí, left bank of Rio Jutaí; 12 - CTGA
994 M170 AM, Igarapé do Jacinto, right bank of the Rio Purus, Tapauá; 14 – FR20, left
995 bank of the Rio Madeira, Lago Xadá; 15 – CCM251, Lago Matupirí, Rio Madeira; 18
996 – MNFS1019, 1020, and 1361 Ocidente, right bank of the Rio Juruá, Acre. Shaded
997 yellow is the hypothetical distribution of *Cebuella* aff. *pygmaea*. Shaded pink is the
998 hypothetical distribution of *C.* cf. *pygmaea* + *C.* cf. *niveiventris* (original shapes
999 downloaded from IUCN (International Union for Conservation of Nature) 2008.
1000 *Cebuella pygmaea*. The IUCN Red List of Threatened Species. Version 2017-2 (as
1001 per Van Roosmalen and Van Roosmalen, 1997).

1002

1003

1004

1005

1006